AD641748

TT66-62576

THE PROBLEM OF FERTILIZATION IN IXODES TICKS

TRANSLATION NO. 1385

June 1965

UNITED STATES ARMY
BIOLOGICAL LABORATORIES
FORT DETRICK, FREDERICK, MARYLAND

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[Following is the translation of an article by I. I. Sokolov, Embryology Faculty of the Leningrad State University, published in the Russian-language periodical Zoologicheskiy Zhurnal (Zoological Journal), 1956, Vol XXXV, No 4, pages 511-528. Translation performed by Sp/6 Charles T. Ostertag Jr.]

In spite of the fact that Ixodes ticks, due to their important economical significance, have served and are serving as the object of numerous investigations, they, nevertheless, prove to be insufficiently studied in several respects. For example, many stages regarding questions of their fertilization remain unclear up until the present time or are explained in different ways by different authors.

During our investigations on the spermato- and ovogenesis of some Ixodes ticks (Ornithodoros papillipes, Dermacentor marginatus, and others), we were able to make a number of observations which have a relation to the phenomena connected with fertilization in them, and in our opinion may be of a certain interest, though all of them are not conclusive.

1. SPERMATOPHORE FERTILIZATION

As is known, in the Ixodes ticks, at the end of spermatocytogenesis, the spermatids convert into large spermatic elements of a very unusual structure. Various authors call them by different names: "spermatids", spermatozoa", "young spermia", "immature spermia", "prospermia", etc. Emanating from the work of O. Tuzet and J. Millot (1937), who explained that in the structure of these elements a very insignificant part falls to the share of the spermium proper, which in their opinion has all the traits of a typical tailed spermium (the truth of which cannot be agreed to unconditionally), we consider that they should be looked at as "carriers of spermia", as spermatophores. Below, we will call the spermatic elements found in the reproductive tract of the male "prespermia", and those found in the reproductive tract of the female "spermia".

Generally, prespermia have the form of very long tubes with one-half pushed into the other half, similar to the turned in finger of a glove (which in the opinion of K. Samson, 1909, achieves a considerable economy of space). The spermium, itself (the nucleus), is located on one side, at the end of the tube. In such a form the prespermia gradually pass into the seminal duct and usually form voluminous spherical packets there, which often include hundreds of prespermia each.

The male sexual apparatus is equipped, at its excretory part, with a complex set of additional glands. One of their functions is sharing in the formation of the main capsule-form receptacles of sperm--the spermatophores. With the help of these the transfer of the sperm into the reproductive tract of the female takes place. During mating, which has been observed by many authors, the male crawls under the female and with its tarsal claws hooks behind her anterior legs in such a way that their ventral surfaces are adjacent to each other. From the porus genitalis of the male the spermatophore is exposed, which it seizes with its chelicerae and transfers it to the porus genitalis of the female.

Spermatophore fertilization has been known for a long time (S. R. Christophers, 1906; G. H. F. Nuttal and C. Merriman, 1911, and others). It received a more detailed clearing up at a relatively recent time (Ostroumova, 1936; Pavlovskiy, 1939; G. G. Robinson, 1942, and others). Nevertheless, a number of obscurities and differences of opinion remain among authors, both in regard to the structure of spermatophores in general and on the problem concerning the source of origin of their membrane and several others.

According to Academy Member Ye. N. Pavlovskiy (1939) it is necessary to differentiate the external spermatophore which the male attaches to the reproductive tract of the female and which hangs loose after copulation in the form of a globule from the porus genitalis, and the internal spermatophore, found already in the female, or the spermatodose -- based on a similarity with the spermatodoses of some insects.

In representatives of the genus Ornithodoros, the external spermatophore in the filled condition is a round body with a cervix, inserted in the porus genitalis or connected with it. The cervix of the spermatophore is wedged in the vagina and promotes the retention of the spermatophore in place. After the transfer of the contents into the uterus, the empty spermatophore remains for some time in the genital rima, covering the outlet from the sexual tract. After falling off the spermatophore the porus genitalis remains gaping to a certain degree (Troitskiy, 1936).

Similar data was obtained for various species of the genera Ixodes (Pavlovskiy, 1939) and others. From the external spermatophore attached to female, the contents enter the uterus. A membrane appears around it and again a packet is formed -- the spermatodose, differing from the spermatophore in form, though it is also round or oval and equipped sometimes with a long and sometimes with a short cervix or "spout". "The walls of the spermatodose are structureless, they represent a hardened secretion, apparently from the accessory sexual glands of the female and in comparison with the walls of the spermatophore appear thinner and more delicate" (Ostroumova, 1936).

C. G. Robinson (1942) finds other connections in Ornithodoros moubata. The final spermatophore, formed at the expense of secretions from the accessory glands of the male, have, according to his data, a more complex structure and consists of a pyriform part, or bulb, a prolonged cervix and two capsules connected with the latter by short canals. The mechanism for introducing sperm is quite complex. Before insemination itself, only the bulb, filled with a colorless liquid, is put out from the male porus genitalis. Then the capsules and cervix, filled with "spermatids" and still located in the ejaculatory duct, are pushed with force into the bulb, which due to this is stretched considerably and becomes white and dull. The lips of the male porus genitalis compress the small sector of the cervix remaining on the outside of the bulb. Then the male grasps the spermatophore, holding it beyond the cervix with its chelicerae, and inserts the proboscis in the vagina of the female. The bulb remains on the outside and after relaxation of the male's grip, the tension of the bulb compels the latter to contract and eject the capsules into the reproductive tract of the female. After contraction, the bulb dessicates into a flat disc, and the "spermatids" are carried out of it through the cervix into the capsules, which now fill up the uterus, pushing against the lower part of the appropriate oviduct. The remainder of the connecting piece of the cervix in each of both capsules contracts and twists (capsule closure), leading to complete closure of the capsule.

Somewhat by itself stands the data of E. Warren (1933) for Haemaphysalis leachi, in which a peculiar spermatophore ribbon is formed. At first tubular "prospermia" are situated along the axis of the impar section of the ejaculatory duct, forming a long compact cord. Then the sperm leaves the body of the male in the form of a long ribbon. Modeling of the sperm into a ribbon is carried out within the vestibulum. However, Warren did not observe the actual transfer of the spermatophore ribbon to the female. After entry of the spermatophore ribbon into the uterus, the latter enlarges greatly and its walls thicken; prior to this, the cuticular lining is strongly folded, is straightened out, and with the subsequent increase of the uterine cavity comes loose from the walls of the latter and forms a sack, lying freely in the cavity and containing the sperm within itself. Later, the cuticle breaks up and the sperm lies directly in the uterine cavity. Apparently, the ova are fertilized during passage through the uterus, where they arrive from the ovary by means of a rupture in its wall, since oviducts are lacking here (!) according to Warren. In many respects, the facts reported by Warren are in need of verification.

If, in this manner, spermatophore fertilization is a characteristic peculiarity of Ixodes ticks, then the problem concerning by which method the spermatodose is formed inside the female or more precisely, what is the origin of the membrane surrounding the sperm, cannot, as we just saw, be considered fully resolved.

In our investigations on the history of the development of germ cells in Ixodes ticks, we were not able to pay special attention to the process of spermatophore fertilization, therefore we report here only about some observations made in passing, pertinent only to internal spermatophores or spermatodoses.

At our disposal were more than two dozen female Ornithodoros papillipes, obtained thanks to the courteous cooperation of Academy Member Ye. N. Pavlovskiy and investigations at a different time of the year, for the most part after starvation for a different number of months. In the majority of these from one to five spermatodoses were found in the cornua of the uterus in a specimen, while there were cases where three spermatodoses were found in one cornu and none in the other.(1)

In a living condition the spermatodoses, which are separated without particular difficulty, have a fairly true spherical form of a thin-walled sac with a diameter from 0.8 to 1.10 mm, more rarely 0.6 mm, filled with sperm. From the wall of the sac a short tube-like spur branches out which is oriented sometimes perpendicularly, sometimes obliquely to it and corresponds to the "spout" referred to by Ye. N. Pavlovskiy. With a spermatodose of particularly large dimensions, due to the limited space within the cornu of the uterus, it may take a lengthened or bean-shaped form, etc. (figure 1, in the inset).

In one case we observed a more complex spermatodose. It was divided by two constrictions into three branches of approximately the same size and round form, each of which, most of all, must be looked at as an independent spermatodose. Between the first and second branches there was a wide connection since the constriction here was not so sharply expressed. From the second branch a finger-like blind branched out which corresponds to the "spout" of the spermatodose. The sharply detached third branch was connected with the second by means of a short narrow canal which should be considered as the "spout" of the third branch. Whether here there was some unusual case of spermatodose formation or maybe the stated branches should be looked at as individual "capsules", from the point of view of Robinson's concepts -- we will not take upon ourselves to judge.

The separated spermatodose possesses a thin, clear wall for the most part devoid of a particular structure. But dispersed sectors are observed on the wall, sometimes in the form of small stages, sometimes in the form of narrow strands which reveal an unclear structure consisting of

(1) A. D. Lees and I. W. L. Beament, 1948, observed up to 12 spermatodoses in O. moubata.

haphazardly grouped cells of various size, giving it a somewhat frosty appearance. This closely suggests the similar structure of the cover surrounding the clutch of eggs of some water ticks (for example, Limnesia maculata, Piona longipalpis) (Sokolov, 1925). However, the similar "frosty" nature of separate sectors of the spermatodose membrane points to its formation by means of the secretion of some rapidly hardening substance.

We also investigated the fixed material in microscopic sections through the entire uteri with spermatodoses included in them and through isolated spermatodoses. Fixation (Buen and Navashina fluid) causes, for the most part, a contraction of the walls of the spermatodose which form numerous folds and creases, so that the contours of the spermatodose sometimes becomes as if strongly jagged and wavy, and its form -- distorted.

The contents of the spermatodose represent a usually compact mass of a large amount of more or less evenly distributed spermia, lying here without any orientation and tightly interwoven with each other (figure 1). In the intervals between them there is a particular substance of a protein nature. It stains very strongly with iron hematoxylin, with an insufficient differentiating with alums it appears black or gray, forming a dark background on which the spermia which are lighter and intersecting in various directions are clearly projected (figures 1 and 2, in the inset).

The uniform distribution of spermia within the membrane of the spermatodose is sometimes disrupted due to the accumulation at some spot of fluid forcing the spermia back to the periphery. Similar pictures are, without a doubt, the result of osmotic influences caused by fixation (figure 2).

On the problem concerning the origin of the spermatodose membrane, as was already said, the opinions of authors are different. From the point of view of Robinson's concepts (1942) concerning the structure of the spermatophore, the problem is resolved relatively simply. The membranes of spermatodoses correspond to the walls of capsules, appearing as parts of a complexly constructed spermatophore which have formed from the very beginning in the male reproductive tracts with the assistance of their supplementary glands, and introduced inside the body of the female together with the spermia contained in them. The spur, named "spout" by Academy Member Ye. N. Pavlovskiy will apparently then correspond to the short canal joining the capsule with the cervix and then closing.[2]

M. V. Ostroumova (1936) and Academy Member Ye. N. Pavlovskiy (1939) consider "spermatodes" as a new formation, different from the external spermatophore and formed within the female reproductive apparatus, as a result of secretions of part of it which is not yet accurately known. Both authors are inclined to attribute such a significance to a pair of small additional sexual glands of a tubular type flowing into the most

Footnote 2. Whether the results obtained by Robinson after their publication are confirmation or are in question remains unknown to us.

rudimentary unpaired thin-walled branch of the uterus (see above). However, a similar assumption, in our opinion, is little probable, on the one hand by reason of the small sizes of the glands mentioned, and on the other hand, because of the site of their inflow, located at a considerable distance from the site of distribution of the spermatodoses.(3)

It is also possible to consider as a possible source for the formation of the spermatodose capsule as the wall of the uterus itself (both of its unpaired section and the cornua). Actually, the pictures obtained by us in microscopic sections through the terminal part of the genital apparatus of O. papillipes indicates an intensive secretion by the uterine epithelium. The latter seems strongly hypertrophic (figures 1 and 3). Its basal part has the form of a continuous plasmatic layer with nuclei preserving their small sizes. The entire remaining part of the epithelium constitutes a wide secretory zone. This zone usually is continuously occupied by "drops" and "tiny lumps" of the secretion, distributed in empty cavities formed as a result of fixation. The "drops" have an irregular form and are very diverse in size, most of the time considerably exceeding the size of nuclei. Apart from this, in many sectors of the uterine cavity there is observed an accumulation of a substance, strongly stained by iron hematoxylin or aniline blue, adjoining close to the inner surface of the epithelium (figure 1, above). This substance, in our view, undoubtedly represents the secretion of the uterine epithelium. In places it may come in contact with the spermatodose membrane.

On the basis of what has been said, it seemed to us at one time quite plausible to consider that the secretory activity of the uterine epithelium causes the formation of the spermatodose capsule. However, other observations compel doubts in such a possibility. On the one hand, we find that free secretion is distributed very unevenly in the uterine cavity; in several places it forms a very considerable accumulation, in others it is distributed in a more or less thin layer, and finally in some places it is lacking completely. On the other hand, simultaneously with the presence in the uterine cornua of spermatodose with a well expressed membrane, there are often observed voluminous dense congregations of spermia, directly adjoining to a great extent with the walls of the uterus, but in spite of this, devoid of any traces of internal membranes. As regards the actual similar congregations of spermia, in our opinion they should be regarded as the result of a rupture of the spermatodose membrane (figure 1, below).

Footnote 3. The function of the stated supplementary sexual glands has not been conclusively explained. The supposition exists (K. Samson, 1909, p.220) that their granular secretion serves as a unique stopper, blocking entry into the vagina after fertilization. Others (A. D. Lees and I. W. L. Beament, 1948,-- in Ixodes; L. E. Robinson and J. Davidson, 1914 -- in Argas persicus) assign to secretion the role of a lubricant, easing the exit of eggs from the vagina.

Thus, in a resume of what has been said there are four opinions as to the possible origin of the spermatodose membrane: 1) the spermatodose membrane corresponds to the "capsule" of the complexly constructed spermatophore (Robinson); 2) it is formed anew due to the secretions of a pair of accessory glands of the female (M. V. Ostroumova, Ye. N. Pavlovskiy); 3) it is formed due to chitinous intima separated from the wall of the spermatheca or uterus (Warren); 4) it is formed anew due to secretion from the uterine epithelium (our proposal which needs verification). Which of these points of view is closest to the truth must be cleared up by specially conducted investigations.

II. Movement of the Spermia

After completion of spermatocytogenesis, the prespermia, still found within the body of the male, represent, as was said, a double lengthy tube, that is, one turned inside of itself. This tube is surrounded by a rather thick layer of a completely transparent substance which D. B. Casteel (1917) named the "gelatinous membrane" (the "wasserreiche Plasmahulle" of Samson, 1909). In the testes, in the places of transfer into the seminal ducts, and also in places in the latter, the prespermia form voluminous dense congregations where they lie in the most diverse positions, being bent in different ways and closely pressed to each other. As a result of this, the contours of their "gelatinous membranes" in microscopic sections of such a type of accumulation receive a polygonal configuration. Only in a few places, small gaps remain between them (figure 4). Undoubtedly, one of the functions of the stated "gelatinous membrane" is the mechanical protection of the tubular prespermia from mutual pressure and friction during their residence and passive movement in the sexual passages of the male.

The walls of the seminal duct are very thin and delicate, as a result of which during preparation in a physiological solution they are very easily ruptured, and the prespermia immediately are as if "spilled out" in various directions from the point of rupture outwards. During this, having been liberated from the mutual pressure, they immediately unbend which indicates a certain elasticity. We, just as other authors, have never observed any other natural movements of prespermia.

The "gelatinous membrane" has a semiliquid consistency and encircles the prespermia with a very voluminous layer in which the prespermia lie freely and can be somewhat dislodged to the side from the general longitudinal axis. With a more prolonged residence of prespermia in the physiological solution, changes are observed in the form of the prespermia, which sometimes may bend into the most fantastic form, sometimes taking a zigzag form, other times taking a form like a penknife, then forming a kind of wide spiral, etc. All these changes are caused by osmotic causes and are connected with the swelling of the "gelatinous membrane", striving as a result of this to take on a spherical form and with this compelling the tubular body of the prespermium to bend passively (figure 5, a, b).

During an even longer observation under these same conditions, vacuoles begin to appear in the "gelatinous membrane". The vacuoles initially form in its periphery, directly under the most delicate surface film, in the form of half moon depressions, and then tear away, are rounded and enter deep inside (figure 5, b).

Often it is possible to observe under those same conditions a partial or a complete slipping down of the "gelatinous membrane" with the prespermium above. In the last case it has the form of a long transparent sausage-like mass, with which the tubular part of the prespermium remains connected only by the very posterior end. During this, even a somewhat partial untwisting of the external tube on the posterior end may take place. Simultaneously, the spermium proper (nucleus), having been disposed up to this in the "gelatinous membrane" on the outside, at the anterior end of the tube, is carried along by it to the rear, as a result of which it arrives at the very posterior end of the tube.

Within the spermatodose, that is, already located in the female reproductive tract, the tube of the prespermia which is turned into itself, apparently under the influence of changed osmotic conditions, finally turned out and acquires the form of an ordinary tube and consequently doubles its greater length.[4]

We, as well as the majority of authors, did not observe the actual process of the tube turning out. Anyhow, judging by a chance picture of a small, partial turning out, and also in accordance with the description of several authors (D. B. Casteel, 1917), the forward, somewhat broadened end of the inner tube begins at this time to gradually be fabricated from the external tube more and more. Simultaneously the edge of the latter, having initially coincided with the position of the anterior end, slips to the rear before complete turning out sets in.

And what is the fate of the "gelatinous membrane" during this? With the finally straightened spermia inside the spermatodose no traces of it are noticed on the outside. As Casteel (1917) suggests, the substance of it gets into the cavity of the tube of the spermium which has doubled its own length. However, keeping in mind the considerable volume of the "gelatinous membrane", it can be suggested that part of this substance is left on the outside. It is completely possible that the previously mentioned structureless substance, filling the spaces between the spermia in the spermatodose, answers namely to the substance of the broken "gelatinous membranes". Of course this remains only an assumption, since in relation to the aforesaid substance, together with this another question can be asked: Is it a secretion of the accessory sex glands of the male during the process of forming sperm and the formation of the spermatophore.

Footnote 4. A more detailed description of the structure of a spermium will be given by us in another place.

In _O. papillipes_ we did not observe any kind of independent movements of spermia within the spermatodoses freshly dissected from the uterus. Just like this they usually remain immobile, even after coming outside into a physiological solution following rupture of the spermatodose membrane. And only in one case was it possible to observe some mobility in them. This consisted of the fact that the very forward end of the spermium made weak, peristaltic-like movements, repeating them about 20 times a minute. During this rotation of the spermium around its own long axis took place. It is possible that here some kind of role is played by the corona of the nipples, found on the most anterior end of the spermium where a derivative of the centrosome is located.

It was possible to conduct a more specific observation of the movements of spermia in _Dermacentor marginatus_. Spermia were taken from the spermatodose or from congregations of them in the oviduct.

After a slight tear of the wall of the oviduct with a needle in a drop of physiological solution on a slide, a ball of sperm immediately flows out of the gap. During this, their anterior broadened ends most of all were protruding on the outside, the thin ends, containing the nucleus of the spermia which is threadlike here, were directed inside the ball. In the spermia leaving the ball which had the form of long straight tubes, it was possible, first of all, to notice a slow progressive sliding movement with the broadened end forward and with a very slight serpentine bending of the entire body. Outwardly, to a certain extent, it called to mind the sliding movement of a gregarine.

Simultaneous with this, the spermia, with their anterior broadened end performed a movement which on the whole represented a combination of a rotary motion with metabolic transformation of a peristaltic nature. To accurately catch these movements is difficult since the form of the widened end during this changes quite rapidly. Figure 6 semischematically presents sketches of four successive phases indicating rotation. The peristaltic motion of the forward end is most easily detected when the spermium attaches it to some object. Under such conditions, successive waves are observed, going from front to rear in a comparatively small sector of the broadened part (figure 6, b). One wave gives way to another, and connected with this is the periodic stimulus-like vibration of all the remaining part of the spermium.

In one of the preparations a small scrap of the oviduct wall was located. Observing the successive movement of an individual spermium, it was possible to observe how the latter diverged from a direct path and began, first, to bend its broadened end in the direction of the scrap of oviduct and then straighten itself out, becoming perpendicular to the surface of the latter. In the course of more than three hours, while observation was going on, many spermia, by precisely the same path became

attached to the surface of a fragment of oviduct. It seems to us that this points to the presence here of a certain thigmotactic or chemotactic response. The possibility isn't ruled out that this attachment is made easier by a certain stickiness of the tip of the spermia itself. After this, the spermia anchors itself on the surface of the fragment, easily immersing in it and continuing to perform rotary movements. By following the conduct of individual spermia, it was possible to obtain ideas concerning some peculiarities of the specific movement. Thus, for example, in one case the spermium stuck its broadened end between a small lump on the oviduct and some large cell; the rear end for some reason remained in place. This led to the fact that due to the rotation the entire long body of the spermia from time to time was bent in an arc similar to the rotating cord of a child's jumping rope. Only these movements occurred with jerks, after which the spermium straightened out each time. The jerks followed one behind the other with a specific rhythm of 22-24 jerks a minute.

In those cases, when the rear end of the spermium was completely free, the rotation of the front end often caused the bending of the entire spermium in a loop, after which it again rapidly straightened out. A number of other analogous observations were made.

In the spermia which were penetrating externally into the tissue of the oviduct fragment, the forward end did cease the rotary-peristaltic movement. This led to the fact that all the remaining part of the spermium behind the broadened end also gradually was drawn passively into the tissue similar to the penetration of a corkscrew into a cork. Due to the great flexibility of spermia for completion of the stated process of implantation, they take on a curved, odd form -- sometimes in the shape of a flat spiral or a serpentine tape, and sometimes in the form of a flat ball, etc., as is shown in the sketches (figure 7).

Many authors make mentio·· of the sliding movement of spermia of Ixodes ticks, but almost none of them have studied it in particular detail, and it is described in different ways. S. R. Christophers (1906), while observing the sliding movement of spermia of O. savignyi with the thickened end forward, correctly considered the latter the "small head" of the spermium.

Based on the observations of Samson (1909) in O. moubata, the movements of spermia were the same serpentine-sliding movements which we observed in O. papillipes and D. marginatus. But Samson notes and describes a small appendage (fliederblattformiger Fortsatz) on the rear end of a spermium. From it into the tube goes a braid (flagellum), and on it there is an elongated nucleus. "This entire branch moves in a live spermia, as is apparent with strong magnification, leaning first to one, then to the other side. During this, the nucleus moves together with it, and the flagellum inside the tube performs jerking movements",

(K. Samson, 1909, page 488). No doubt the author was dealing with the passive movements of the designated parts, caused by rotation of the entire spermium.

The Indian biologist G. P. Sharma (1944), who not too long ago investigated spermatogenesis in Argas persicus, Hyalomma aegyptium and Rhipicephalus sanguineus, also observed the active movement of spermia, taken from a female, in a normal physiological solution of NaCl. They performed progressive movement along a straight line, which is called "The peculiar rotary movement of the large ring-shaped centrosome", located at the very anterior end of the spermium which is mainly in agreement with our observations. But together with this, Sharma admits, with reference to Casteel, that granular mitochondria, distributed by longitudinal rows under the very surface of the tube, also assist the peculiar movement of the spermia.

It is not likely in the given case that there is a basis to ascribe to mitochondria any role in the movement of spermia.

III. THE FATE OF SPERMIA IN THE OVIDUCTS

The spermia may reside within the spermatodose for the course of many months without losing their viability. But sooner or later they should vacate the spermatodose and get into the reproductive tract of the female in order to carry out fertilization of the eggs.

With regard to the manner that the spermia come out of the spermatodose, there also is no complete accord among authors. In the spermatodose of O. savignyi, Christophers (1906, table IV, figures 5 and 20) describes and depicts a full tubiform spur through which the spermia leave when it is compressed. Exactly so, Academy member Ye. N. Pavlovskiy (1939) considers that in Ornithodoros the "spout" mentioned above serves for the exit of spermia. Such an opinion is adhered to also by M. V. Ostroumov (1936). According to him, in O. papillipes and O. lahorensis the spermia are forced out into the oviduct through the long cervix of the spermatodose due to a shortening of the distended sinewy walls of the uterine cornu and are moved further forward through the oviduct as a result of a contraction of its walls.

Such a view to a certain degree is confirmed also by our observations on the spermatodose of Hyalomma detritum. In microscopic cross sections, the spermatodose had a very regular round form, around 0.5 mm in diameter, and its "spout" seemed in the shape of a long (around 3/4 the diameter of the spermatodose), narrow, straight tube, bent on the very end at a right angle and having thicker walls than the spermatodose itself. In the lumen of the tube a certain number of spermia are found. Their presence can be ascertained by their dark stained filiform nuclei. In the given case the spermatodose was surrounded by a very heavy mass of a particular substance which fills the entire cavity of the seminal receptacle and apparently corresponds to the "membrane" which Samson depicts

in his drawing of a cross section of the different parts of the reproductive apparatus in Ixodes ricinus (K. Samson, 1909, table XI, figure 17, H). In this substance it was also possible to differentiate here and there, filiform formations strongly staining with hematoxylin which evidently correspond to the nuclei of the spermia. It is possible that here we have before us spermia which were just leaving through the "spout".

In other cases, for example in the spermatodose of O. papillipes, the "spout" develops more strongly, according to our observations, is often tapered down on the end and moreover it is often closed here. Still keeping in mind the very narrow diameter of its lumen, we propose that the "spout" can hardly be looked on in general as a special attachment for the outlet of spermia. If it is not closed on the end then they of course can exit through it, but this apparently happens far from often.

In view of the great delicacy of the spermatodose walls, it is more preferable to suggest that the outflow of spermia takes place simply by means of a rupture of these walls in some place, possibly due to constriction of the muscles of the walls of the uterus or seminal receptacle. This was actually observed, for example, by Robinson and Davidson, 1914, in Argas persicus where the thin membrane of the spermatodose splits and the spermia are liberated and pass into the uterine cornua and oviducts.

According to Robinson (1942), in O. moubata the "spermatids" (prespermia) mature (that is, reverse -- I. S.) in the capsules within the uterus in about 5 days at 30° and leave in the form of "spermia" through the walls of the capsule. This may take place when the female is feeding or when the eggs enter the uterus.

It was already pointed out above, that in O. papillipes, besides the spermatodose, we often simultaneously observed in the cavity of the uterine cornua, voluminous congregations of spermia lying freely, that is, not surrounded by any kind of membrane (figure 1). Apparently, we have here before us, spermia which had already come from the spermatodose into the uterine cavity.

Before going further, it is necessary to touch lightly on the structure of the reproduction apparatus in O. papillipes. Out of the ovary, from each side leads a thin, tubular, usually strongly twisting oviduct, each of which goes to a small but clearly expressed widened section which in general is oval shaped. Beyond it, the oviduct again acquires the shape of a narrow canal, forming during the course of its passage the characteristic bend in the area of the uterine cornua, and finally connecting with the corresponding uterine cornua. Academy member Ye. N. Pavlovskiy (1939) who gives a description and sketch of this same apparatus, considers the referred to widened section of the oviduct as the initial part

of the uterine cornua, as its summit, beyond which follows the narrow and finally the wide part of the cornua. On the basis of our observations of significant histological differences in the structure of the walls of the three sections referred to, we accept as the uterine cornu only the most distal wide section, which in our preparations had the constant appearance of a thick-walled sacculate tube (figure 8, in the inset). At its proximal end the uterine cornu transforms into a narrow canal which we name the distal section of the oviduct (figure 8). The latter, in its turn sharply transforms into a widened section of the oviduct, beyond which follows an also clearly limited, proximal section of the oviduct. In the semischematic drawing of Ye. N. Pavlovskiy (1939, figure 16) the place of transformation of the proximal section into the widened one is depicted quite correctly. In our preparations, in longitudinal cross sections through that same place, the end of the oviduct contiguous to the widening presses into the latter in the form of a sharply expressed fold, forming a type of valve (figure 9, in the inset), which may play an important role in the passage of eggs or in the passage of spermia in the direction of the ovary, regulating these transfers.

As is known, in O. papillipes, both hungry as well as freshly fed females mate. Besides this, there may be repeated copulation, sometimes with the same male (Troitskiy, 1936; G. G. Robinson, 1942; and others). But laying of the eggs is carried out only after the female becomes engorged. Therefore, depending on the situation, the interval of time from the moment of spermatophore fertilization up until egg laying may vary considerably. We do not have specific information on how long the entirety of the spermatodose, which is formed following mating, is maintained and when the spermia leave it.

In our examples of O. papillipes which had gone hungry for several months, along with the spermatodoses we constantly found spermia both in the proximal section of the oviducts as well as in their widened section. However, in the distal section they were lacking, which apparently showed that this entry took place a long time prior to the moment of observation. Besides this, accumulations of spermia were often observed directly in the cavities of the uterine cornua. It can be proposed that with the presence of several spermatodoses, their rupturing apparently takes place in some kind of a sequence. The very first of them probably ruptures comparatively rapidly after its formation and the spermia which exit from it pass from the uterine cavity into the various sections of the oviducts. If soon after this, the female becomes engorged, then the completion of the growth of the egg cells takes place and together with this their fertilization. If a condition for the nourishment of the female is not found immediately and conditions of more or less prolonged starvation set in, then the spermia which have already arrived in the oviducts cannot be used for fertilization. Subsequently their fates may be diverse. This we will go into in detail below.

The remaining spermatodoses found in the uterus do not lose their entirety following prolonged starvation, and thereby preserve the viability of the spermia included in them for some time, until conditions are favorable for carrying out fertilization. A certain sequence for the rupture of the spermatodoses should apparently be manifested also during the period of laying when the eggs are laid in individual portions after specific time intervals of a period of several days (Troitskiy, 1936; G. G. Robinson, 1942, and others).

The prolonged residence of spermia in the proximal section of the oviducts and also in their widened sector, caused by this or that reason is not without aftereffects, and on the one hand it exerts an influence on the walls of the stated sectors of the oviducts, and on the other it is reflected in the fate of the spermia themselves. Up until now attention hasn't been paid to this and only by Robinson and Davidson (1914) do we find the following brief observation: "The lower wider section of the oviducts becomes expanded due to the presence of spermia, and the histological parts of their walls are disintegrated to a considerable degree".

Our observations are in regard to O. papillipes and D. marginatus. Since at our disposal there were only several series of microscopic sections through fixed material, then it was difficult to establish the entire sequence of events of the changes described below, however, we think that the basic substance of the processes grasped by us is correct.

The initial and main moment here is the penetration of the spermia into the walls of the oviducts. It is possible that some role in the first impulse during this is played by the sterotaxis reaction of the spermia, which was mentioned above and which, in the given case, leads to their orientation with the forward end perpendicular to the internal surface of the walls of the oviducts. After this, their implantation begins. Depending on the overall number of spermia in this or that sector of an oviduct, there is a variation in the number of spermia which penetrate into its wall. The mutual grouping of spermia is also variable.

Figure 10 depicts a part of a cross section of the proximal section of the oviduct of Demacentor. A large number of spermia are visible which have directed their anterior ends approximately at right angles to the oviduct wall and have implanted in it. They have grouped in small piles. Some have already reached the thin muscle layer of the wall, some are found halfway there. The posterior ends, where the filamentous nucleus of the spermium is enclosed, are freely hanging out in the lumen of the oviduct. The appearance of the spermia is completely normal, apparently speaking for the fact that their implantation took place relatively recently.

Figure 11 clearly shows that the spermia actually penetrate into the cells of the oviduct and not in the spaces in between them; in the

apical section of one cell, five spermia cutting across are apparent, and in another one -- four. The nuclei of the oviduct cells in this case are hardly changed, perhaps only slightly enlarged. But then the apical sections of the cells themselves are stretched out into long spurs of an odd form, hanging in the lumen of the oviduct. In their plasma, a rather sharply expressed fibrillar structure was developing (figures 10 and 11) which, maybe, indicates the beginning of degeneration of these sections. At any rate these pictures clearly show the peculiar reaction of the oviduct cells to the implantation in them of such large formations as spermia.

Figure 12 (in the inset) presents a cross section through the proximal sector of the oviduct of another example of D. marginatus with an especially profuse congregation of spermia in it. A huge number of the spermia formed a rather normal, wide and very dense bundle implanted in the wall of the oviduct for the extent of almost 1/3 of its surface. The corresponding sector of the wall experienced here more significant changes already, keeping the form of a very narrow strip at the same time that apical ends of the cells were disintegrating; the nuclei of the latter were preserved and changed little; the nuclei of neighboring cells were noticeably enlarged and acquired somewhat incorrect contours.

Just now the described picture speaks as if there were some kind of orienting influences on the spermia on the part of the oviduct walls. But, besides this, it is necessary to also note the capability of the spermia to group in true dense bundles, made up of a very various, and sometimes in individual cases, a great number of them.

As a result of the more prolonged residence of the spermia in the walls of the oviduct, the cells of the latter in the corresponding site are finally destroyed so that the thin muscle layer is all that is preserved (figure 13).

All that has been said compels the proposal that the spermia secrete some kind of lytic substances which act on plasma in a dissolving manner. Often it can be seen that a spermium which had penetrated into the plasma lies as if in a hyaline vacuole, which apparently corresponds to the initial phase of dissolving the cytoplasm. In other cases we observe various subsequent phases of dissolving the oviduct cells, beginning with their apical parts. If the spermia manage to penetrate into the basal sections of the cells, then the disintegration of the latter may lead to the fact that individual nuclei together with a small portion of the cytoplasm surrounding them may fall into the lumen of the oviduct (as is depicted, for example, in figure 13), where they then degenerate.

It is interesting to note that the nuclei of oviduct cells, in certain cases display very considerable changes. On the one hand, they are strongly increased in volume in comparison with nuclei from other

sectors of the oviduct which do not contain spermia. On the other hand, they simultaneously take on irregular contours (figure 14), and become lacinated. By this, they increase their overall relative "active" surface, similar to the nuclei of various strongly functioning cells, such as, for example, the feeding cells in the ovarian tubes of insects, etc. The nuclei have a roughly granulated structure and contain a various amount of strongly staining iron hemotoxylin "lumps" of different sizes (figure 14).

What is the significance of all these occurrences? The material at our disposal is insufficient for any conclusive results. Nevertheless, a number of interesting assumptions are suggested.

If you recall that in the reproductive tracts of the males, the prespermia (still in the form of turned-in tubes) are residing in an immobile state, forming dense congregations, and subsequently (already in a turned-out condition) form analogous dense congregations in the spermatodoses within the body of the female, then it follows to suggest that this is never expressed in other cases, that in such a condition the volume of substances in them is strongly decreased and for maintaining their viability they do not require particular sources of energy. They may reside in such a form for months. But after they arrive in the oviducts they become independent, they display a capability for active movement and for maintaining their viability for a long time--and this is connected with the biological characteristics of Ixodes ticks -- they require some sources of energy, and, in our opinion this source is the cells of the oviduct walls. Having embedded in these cells, the spermia, to some or other degree, break them down, and apparently use the products of this breaking up for nourishment.

Pictures, similar to those depicted in figures 10 and 12 in which a specific orientation is conspicuous, when the anterior widened end is located within the cell and the posterior end, which is strictly bearing the spermium (nucleus), is freely projected in the lumen of the oviduct, pointed initially to the concept that such an orientation in all probability was connected somehow with the process of fertilizing the eggs. We suggest that during the specified orientation, the eggs, passing by the spermia through the oviduct, come in contact with the spermia (nucleus) proper located on the posterior end and at this moment the spermia enter the egg; the spermiophores (tubes) deprived of their spermia, remain in place and apparently later somehow or other degenerate. Also in favor of such an assumption are the indications of M. V. Ostroumova (1936) which state that in O. papillipes "fertilization takes place in the oviducts".

Actually, fertilization of eggs in Ixodes ticks takes place by a far more complex method. Mainly, the opinions of authors are different in

regards to the designation of the section of the female reproductive apparatus where fertilization takes place. Further, we hardly know anything about how the process itself takes place. Finally, closely related to this is the question of when and how the shell of the eggs is formed.

According to Samson (1909), in Ixodes the "spermia" pass to the ovary where fertilization takes place; however, the author does not report any further details. Christophers (1906, page 43) also speaks of detecting spermia in the ovary of O. savignyi. He suggested that fertilization must take placé here often. We didn't observe spermia in the ovaries of O. papillipes or D. marginatus with the exception of one case of detecting a single spermium.

Robinson and Davidson (1914) in their monograph on Argas persicus point out that part of the wall of the ovarian tube consists of cells having a glandular form (figure 3) and consider it probable that the latter may secrete the shell of the egg. But since, on the other hand, the authors never encountered spermia above the upper section of the oviduct, and the greatest number of them they observed in the uterus and the lower part of the oviduct, then they assume in as much as fertilization has to take place prior to the formation of the shell, the latter is apparently secreted by the epithelial lining of the oviduct.

Lees and Beament (1948) dispute this assumption, since they observed individual spermia in the lumen of the ovary and also large congregations of them in the upper parts of the oviducts, and they consider that fertilization takes place in the ovary itself (see below), moreover, prior to formation of the shell.

All this compels us to turn to the problem concerning the development processes of the egg itself and of its membrane.

As is known, the deposited eggs of Ixodes ticks are equipped with a dense membrane, or a shell called the chorion by the majority of authors. The details of its structure have been studied only recently. Thus for example, in the eggs of Ixodes ricinus, Arthur (1948) distinguishes the exochorion and the endochorion--an elastic layer, representing an actual "egg shell"; between them is found a lipoid layer which permits them to separate. A vitelline membrane is fitted to the cortical layer. On the very surface of this membrane is found a layer of waxy substance, originating from the Zhene organ.

Lees and Beament (1948) report similar facts about the egg membranes in O. moubata. These authors stress that the surface of the egg is perfectly smooth, without any kind of sculpture, and that there isn't any micropyle. In regards to the problem of egg development, they point out that the egg cells develop from undifferentiated cells, lying in the genital

ridge of the ovary. On the outside, this section is covered with a thin hyaline membrane (tunica propria). With growth, the egg raises this membrane a little and protrudes on the surface of the ovary. When the egg reaches a diameter of 100μ, a stemlet or pedicel is formed. Its walls are formed by one layer of undifferentiated epithelial cells. When a diameter of 500μ is reached, ovulation takes place in the ovary cavity. The last process has not been observed by anyone, but since, as Christophers (1906), who arrived at the same conclusion in his time, also assumes, the growth of the oocyte causes tension of the elastic tunica. Apparently, the pressure of the latter opens the cells of the pedicel and the egg is forced into the cavity. The first appearance of a shell is noted already in oocytes of 25μ and less. As part of the growth of the latter under the tunica, both the overall surface and the thickness of the shell increase. From here, Lees and Beament conclude that the shell must be secreted by the egg cell itself. Therefore, to designate it by the term "chorion", as is applied with insects, for example, is incorrect, since it is formed without the follicle.

However, several obscurities remain in this problem. M. V. Ostroumova (1936) notes that growing egg cells in O. Lahorensis and O. papillipes are surrounded by a distinct follicular membrane, passing on to the pedicel on which the egg cells are setting and in the corresponding drawing depicted several nuclei in the follicle. However, she writes further that in the eggs which are ready for discharge into the oviduct, the nuclei of the follicle are unnoticed since the eggs are provided with a double-contoured chitinous membrane.

In checking these indications in microscopic sections of the ovary of nymph IV and adult O. papillipes, including microscopic sections, treated by Feulgen staining where the nuclei of epithelial cells and pedicels stand out very clearly, we could not detect nuclei in the thin layer surrounding the peripheral part of young oocytes emerging on the surface of the ovary, thus we should rather be attached to the data of Christophers, Lee and Beament.

In view of the dense nature of the shell, the authors named consider it very improbable that the spermia penetrated the egg after the development of the membrane began, and in regards to the problem of fertilization write: "Apparently, fertilization takes place in the ovocytes at a place within the genital ridge, before the rudiments of the shell are settled in the beginning of the growth phase". They cite an analogous case of the implantation of spermia in early oocytes of Peripatopsis (Manton, 1938), though there, their function is supplying the growing oocyte with nutrient material. We could point out yet several cases of early fertilization of oocytes in the beginning of the growth period, for example, in Saccocirrus (O. Buchner, 1914), in Platyhelminthes -- Otomesostoma auditivum (N. Hofsten, 1909), Oxyposthia praedator from Acoela (Ivanov, 1952) and Brachycoelium (G. A. Kemnitz, 1913), and in Rotifera Asplanchna priodonta (O. Storch, 1924). However, in regards to Ixodes ticks this is still only an assumption. It is true that Christophers (1906, page 40) found, in

the protoplasm of eggs of a fixed size, remnants of large "spermatozoids" and even provided a description of an oocyte of O. savignyi having 1/4 the size of a mature egg and containing obvious remnants of spermia (S. R. Christophers, 1906, table IV, figure 10), but since that day these observations have not been confirmed by anyone. Lees and Beament themselves, in spite of thorough research in O. moubata, could not find similar traces in even one of the stages of development of the oocyte. For the present, such negative results have been received by us.

Up until now the actual process of fertilization of the egg cells has not been observed by anyone. There exists only one brief indication by Samson (1909), who, after numerous attempts, succeeded one time in observing, under a microscope, artificial fertilization in Ixodes ricinus. During this, the first part to penetrate into the egg was the nuclear part of the spermia (that is, the posterior end containing strictly the spermia) which is depicted by the author in figure 3. However, it was not seen in later stages so that it remained unexplained if the remaining part of the spermia entered the egg or not.

Stemming from the concept concerning the seed elements in Ixodes ticks as "spermiophores" (see above), in which the inherent spermium (nucleus) occupies only an insignificant part, it can be thought that in the process of conjugation, most of the time the entire spermiophore in general does not take part, but only its nuclear part, the remaining body of the spermiophore remains outside the egg. It may be that the "puzzling formation" of an unknown origin, having the form of a small boat closely adjoining the surface of mature eggs found in the ovary cavity, which Christophers depicted in table IV, figure 7, is just a remnant of a spermiophore.

The more prolonged residence of spermia in various parts of the oviducts, in particular in those cases when the requirement for them is already exhausted or when there is simultaneously a "fresh" reserve of them in the spermatodoses, leads to interesting phenomena, unrecorded up until this time, connected with their degeneration.

In figure 15 (in the inset) a cross section is presented through the enlarged section of the oviduct of O. papillipes. In the lumen of the oviduct two accumulations of spermia are visible which are intersected by a distinct method; part of them are found in contact with the wall. It is conspicuous that in the walls themselves there are very large forms, most of the time with an irregular shape, clusters, surrounded by a void, like a vacuole, obtained apparently as a result of a nodule of protoplasms between them (a result of fixation?). Part of these clusters, following a great enlargement, seemed homogeneous; in another part there was noted a certain structure of a various degree of clearness,

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Figure 1.

Figure 2.

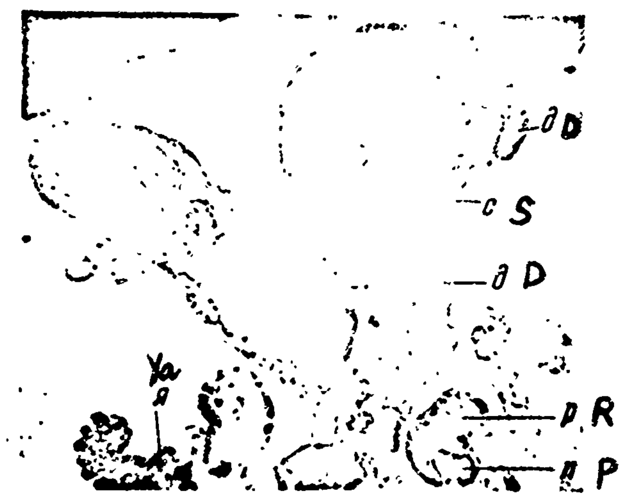


Figure 8.

Fig. 1. Ornithodorus papillipes. Cross section of the cornu of the uterus with a spermatodose; below -- loose accumulation of spermia, above -- accumulation of secretion.
Fig. 2. Ornithodorus papillipes. Cross section through a spermatodose; inside -- an accumulation of liquid.
Fig. 3. Ornithodorus papillipes. Cross section through the cornu of the uterus, in each -- two spermatodoses (s)
d - distal section of the oviduct, r - its widened section, p - its proximal section, ya - ovary.

Figure [redacted] 9

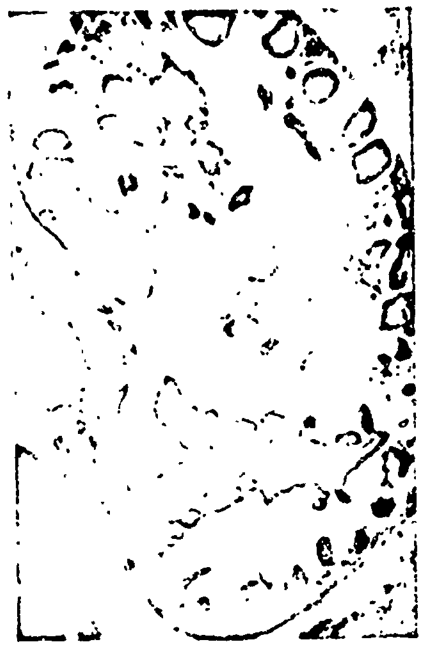


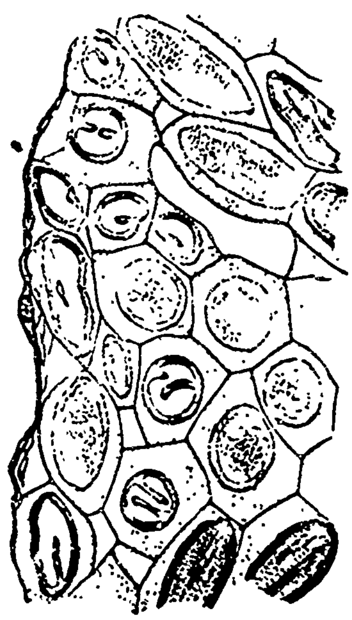
Figure [redacted] 15

Figure [redacted] 12

Figure[redacted] 16

Fig. 9. Ornithodoros papillipes. Cross section through the point of connection of the widened section of the oviduct with its proximal section (valve).
Fig. 12. Dermacentor marginatus. Transverse cross section of the oviduct; on the left -- large bundle of spermia injected into the wall.
Fig. 15. Ornithodoros papillipes. Cross section of the widened section of the oviduct. In the clearance -- two congregations of spermia. In the walls -- spermia completely penetrated and exposed to degeneration and "phagocytosis".
Fig. 16. Ornithodoros papillipes. Cross section of the proximal part of the oviduct. Spermia (the beginning of degeneration) are in the cavity and partially in the walls.

Figure 3. Ornithodoros papillipes. Part of the uterine wall; secretion.



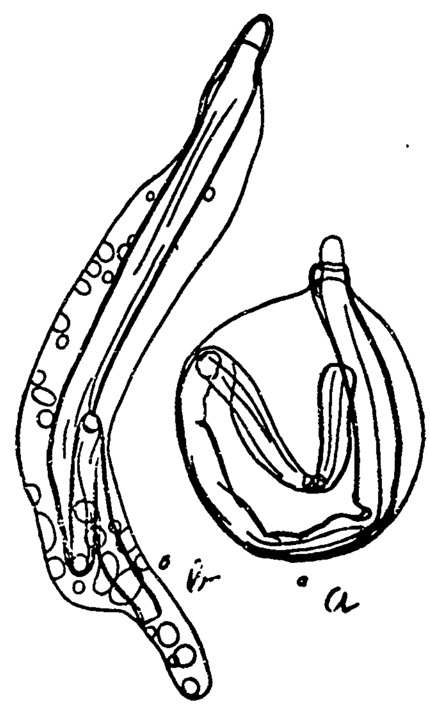


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Figure 4. Ornithodoros papillipes. Cross section through a part of the vas deferens, filled with prespermia equipped with a "gelatinous membrane" (semischematic).

Figure 5. Ornithodoros papillipes. Prespermia from the vas deferens with a swollen (a) and a vacuolized (b) "gelatinous membrane" (sketches from live objects).

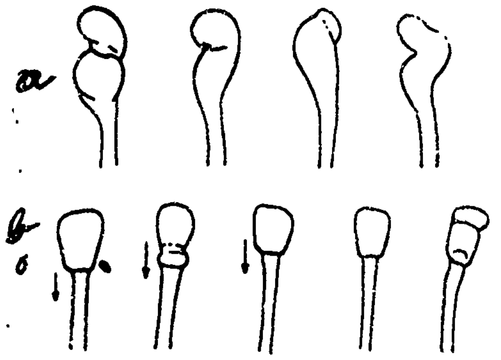

Figure 6. Dermacentor marginatus. **Anterior** ends of spermia from the **spermatodose.** Successive phases **of rotatory (a)** and peristaltic (b) **movement (sketched from live objects).**

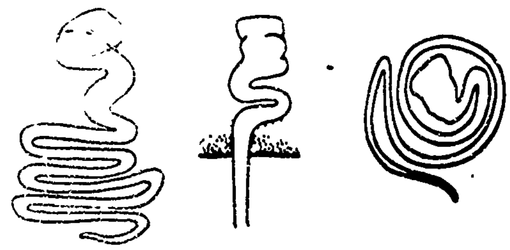

Figure 7. **Dermacentor marginatus.** Spermia which **have** taken **root in the** tissue (sketched **from live objects).**

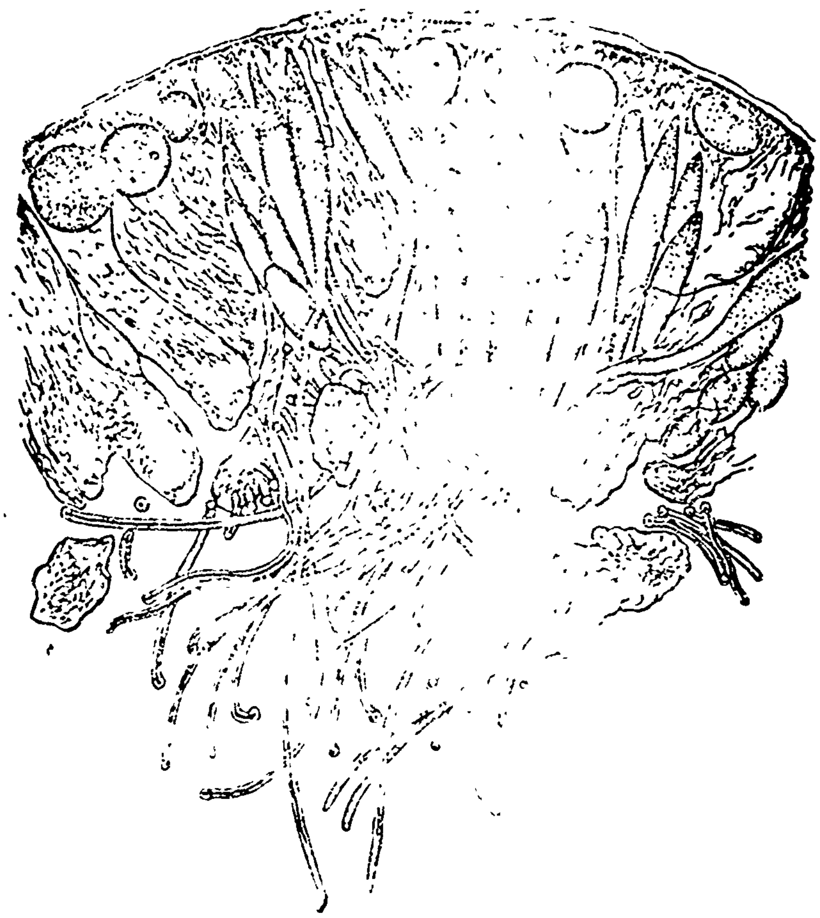

Figure 10. Dermacentor marginatus. Part of the **oviduct wall with spermia which have taken root.** The cells formed spurs with **a fibrous structure.**

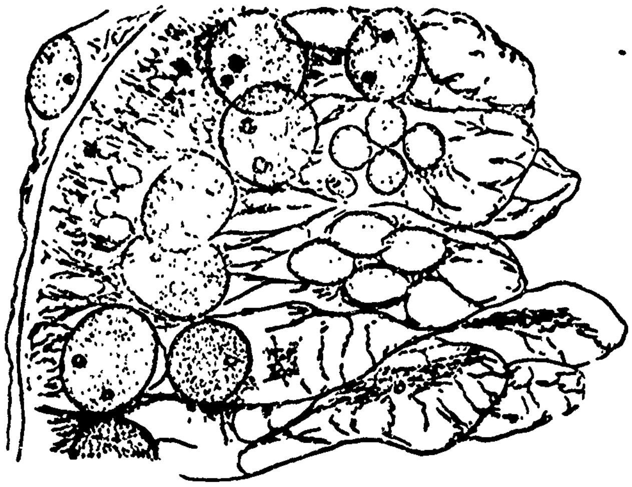

Figure 11. Dermacentor marginatus. Part of the oviduct wall. In the plasma of two cells, spermia transected across are visible.

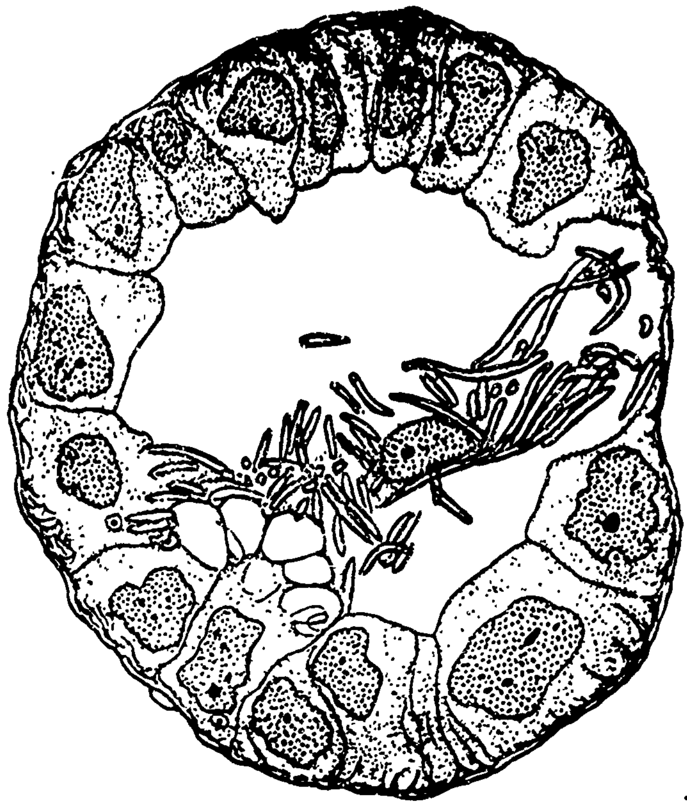

Figure 13. Dermacentor marginatus. Cross section through the oviduct. The destruction of its wall by spermia --in the lumen is a nucleus which has entered.

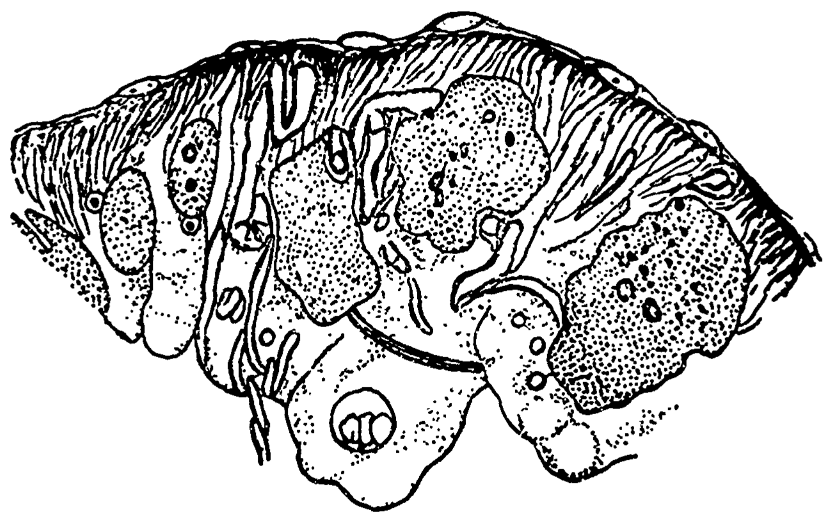

Figure 14. Dermacentor marginatus. Part of the oviduct wall with the injected spermia. The nuclei of the oviduct cells are laciniated and strongly enlarged.